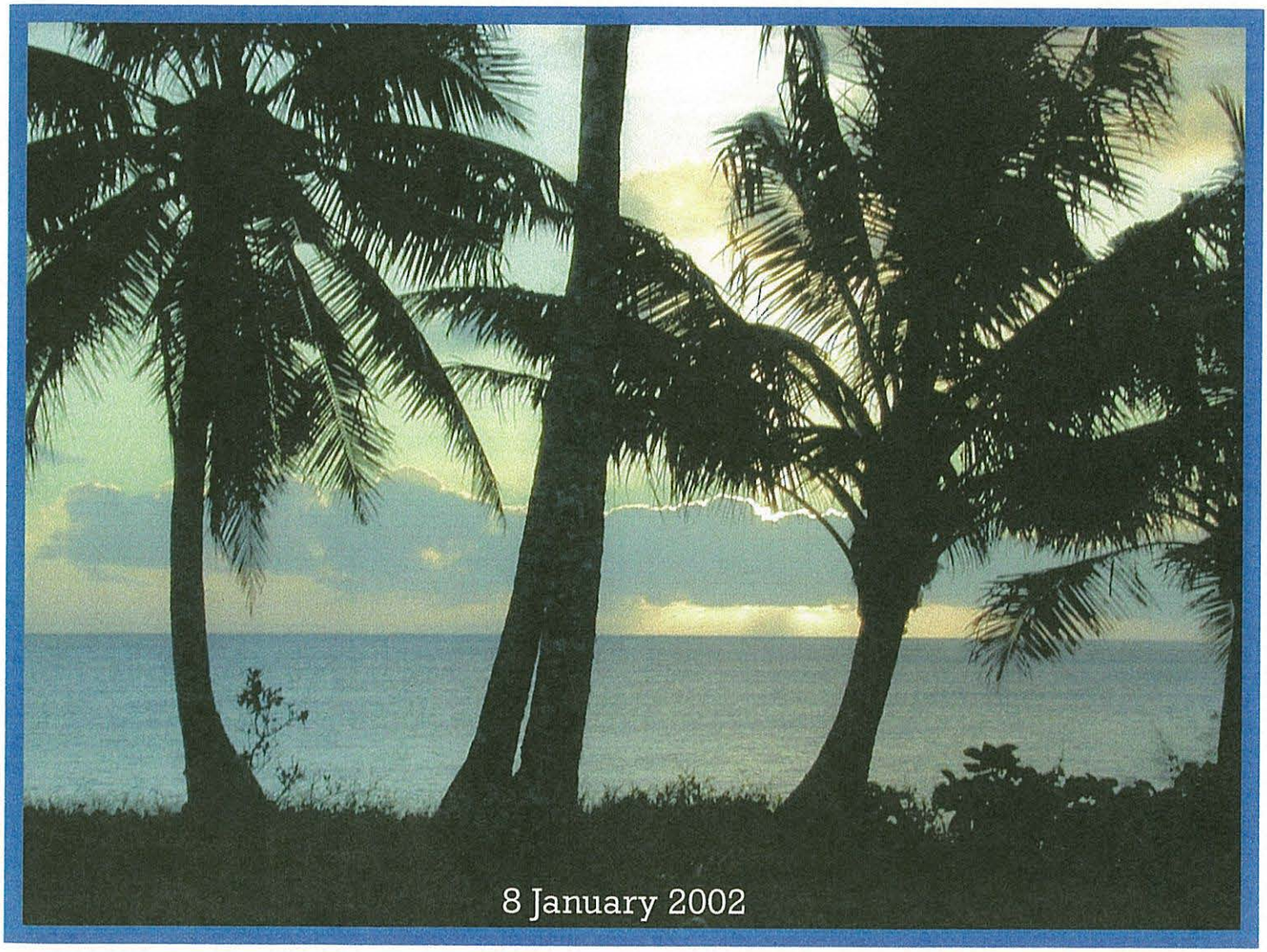
Samoa Joint Cannery Outfall
2001 Tradewind Season
Effluent Bioassay Test Results
October 2001 Sampling



8 January 2002

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TECHNICAL MEMORANDUM

BIOASSAY TESTING – JOINT CANNERY OUTFALL EFFLUENT OCTOBER 2001 SAMPLING

Prepared For: StarKist Samoa (NPDES Permit AS0000019)
COS Samoa Packing (NPDES Permit AS0000027)

Prepared By: Steve Costa
Karen Glatzel

Date: 8 January 2002

Distribution: Carl Goldstein
United States Environmental Protection Agency, Region 9
Peter Peshut
American Samoa Environmental Protection Agency

Purpose

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in October 2001. The testing is required by the NPDES Permits that became effective in January 2001. This is the second required semiannual test required by the current permits and the sixteenth semiannual test conducted since testing for the Joint Cannery Outfall began in 1993.

Study Objectives

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES Permits requires that semiannual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these tests is to determine whether, and at what effluent concentration, acute toxicity may be detected for the combined cannery discharge.

Study Approach

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests conducted in the past. All comments from U.S. EPA have been incorporated into either the sampling/sample handling standard operating procedures (SOP) or the procedures used by the laboratory doing the test. The comments, responses, and SOP have been documented in previous reports.

The permit conditions require that the bioassay tests be conducted with the white shrimp, *Penaeus vannami* (postlarvae). In the event *Penaeus vannami* is not available at the time of the tests the permit specifies the substitute species *Mysidopsis bahia,* which has now been renamed *Americamysis bahia.* For the October 2001 sampling *Penaeus vannami* was not available and *Americamysis bahia* was used.

Effluent samples are collected from the StarKist Samoa and COS Samoa Packing facilities as 24-hour composite samples. The effluent acute bioassay test is conducted using a combined composite effluent sample made up from the effluent samples from both canneries, as allowed by the permit condition. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall into Pago Pago Harbor.

Effluent Sampling Methods

Between 0900 on 25 October 2001 and 0600 on 26 October 2001, 24-hour flow-weighted composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites. Detailed sampling procedures are described in the established SOP for cannery effluent sampling.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at three-hour intervals over a 24-hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times, effluent flow rates, and the relative effluent flow volumes calculated from plant flow records are summarized in Table 1. The relative effluent flow volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL to the testing laboratory. The chain-of-custody form is provided in Attachment I.

Table 1
StarKist Samoa and COS Samoa Packing
24-hour Composite Effluent Sample for Bioassay Testing
October 2001

Grab Sample Number	COS Samoa Packing		StarKist Samoa		COS Samoa Packing Percent of Total Flow	StarKist Samoa Percent of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)		
1	25 Oct 2001 0900	1.20	25 Oct 2001 0900	1.68	4.4	6.2
2	1200	1.20	1200	2.14	4.4	7.9
3	1500	1.20	1500	2.17	4.4	8.0
4	1800	1.20	1800	2.22	4.4	8.2
5	2100	1.20	2100	2.20	4.4	8.1
6	26 Oct 2001 0000	1.20	26 Oct 2001 0000	2.26	4.4	8.4
7	0300	1.16	0300	2.26	4.3	8.4
8	0600	1.16	0600	2.61	4.3	9.6
Total		9.52		17.54	35.2	64.8
Mean		1.19		2.19		

Bioassay Testing Procedures

EnviroSystems, Inc. in Hampton, New Hampshire conducted the bioassay tests from 1 November to 5 November 2001. The testing procedures and results of the bioassay tests are provided in the Laboratory Report included as Attachment II. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA document Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (EPA/600/4-90/027F), August 1993, as the source of methods for conducting the test. The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports.

The test organisms were ≤ 5 days old and salinity was adjusted to 24 ppt. The test temperature was to be held at 20 ±2°C, but actual temperatures ranged from 21°C to

24 °C. The laboratory opinion is that the deviation in temperature had no impact on the outcome of the bioassay test. Demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD) has been discussed and documented in previous technical memoranda describing the first two bioassay tests conducted in 1993. To avoid the effects of the IDOD, which would not be encountered in the field, each bioassay test chamber was continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). The test was renewed at 48 hours and renewal concentrations were aerated prior to the renewal.

Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% as vol:vol dilutions in seawater. Water quality was monitored daily and measured parameters included DO, pH, salinity, and temperature. Total residual chlorine and ammonia were measured. A reference toxicant test using sodium dodecyl sulfonate (SDS) was conducted and results were within one standard deviation of the established laboratory mean.

Results

The results of the bioassay tests are summarized as follows:

Americamysis bahia **Effluent Bioassay.** All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the 96-hour LC_{50} for the effluent tested was 37.52 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was 25.0 percent and the Least Observable Effects Concentration (LOEC) was 50.0 percent. Results on a daily basis are summarized in Table 2.

Americamysis bahia **Reference Toxicant Bioassay.** The reference toxicant test had an LC_{50} of 23.4 mg/l. The laboratory mean is 19.8±4.36 mg/l (based on 130 tests). The test data falls within one standard deviation of the laboratory reference mean, indicating normal sensitivity.

Table 2 StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results - October 2001 Sampling			
Exposure Time	Parameter		
	LC_{50}	NOEC	LOEC
24 hours	46.78%	25.0%	50.0%
48 hours	44.86%	25.0%	50.0%
72 hours	37.52%	25.0%	50.0%
96 hours	37.52%	25.0%	50.0%

Discussion

Table 3 summarizes the results of the effluent bioassay tests for the samples collected in the October 2001 sampling compared to the previous bioassay tests. The LC_{50}, NOEC, and LOEC are within the range obtained from previous reports where *Americamysis bahia* (*Mysidopsis bahia)* was used in place of *Penaeus vannami.*

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for October 2001 do not indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an NOEC of 25.0%, which was observed in October 2001, corresponds to a dilution of 4:1, which is achieved within a second and within 1-meter of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

Table 3
StarKist Samoa and COS Samoa Packing
Combined Effluent Bioassay Results

Date	*Species*	Parameters		
		LC 50	NOEC	LOEC
2/93	*Penaeus vannami*	4.8%[1]	3.1%	6.25%
10/93	*Penaeus vannami*	15.67%	3.1%	6.25%
2/94	*Penaeus vannami*	15.76%	<1.6%	1.6%
10/94	*Mysidopsis bahia*[2]	31.2%	25%	50%
3/95	*Penaeus vannami*	14.8%	6.25%	12.5%
3/95	*Mysidopsis bahia*[3]	10.8%	6.25%	12.5%
2/96	*Penaeus vannami*	>50%	>50%	>50%
2/96	*Mysidopsis bahia*[3]	28.36%	12.5%	25%
3/96	*Penaeus vannami*	44.4%	25%	50%
11/96	*Penaeus vannami*	7.11%	3.1%	6.25%
03/97	*Penaeus vannami*	39.36%	12.5%	25%
09/97	*Penaeus vannami*[4]	12.3%	6.25%	12.5%
06/98	*Mysidopsis bahia*[2]	17.2%	6.25%	12.5%
11/98	*Mysidopsis bahia*[2]	15%	6.25%	12.5%
02/00	*Mysidopsis bahia*[2]	20%	6.25%	12.5%
08/00	*Mysidopsis bahia*[2]	17.1%	3.1%	6.25%
03/01	*Americamysis bahia*[5]	13.8%	12.5%	25.0%
10/01	*Americamysis bahia*[6]	37.52%	25.0%	50.0%

[1]The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.

[2]*Mysidopsis bahia* used as substitutes because *Penaeus vannami* not available: as directed and approved by U. S. EPA.

[3]*Mysidopsis bahia* used in addition to *Penaeus vannami* as described in text of technical memorandums reporting test results. Only one species is required by the permit conditions.

[4]Stage 1 (3 mm) *Penaeus vannami* were used for testing because older Stage 7 and 8 (8-10 mm) *Penaeus vannami* were not available.

[5]*Mysidopsis bahia* renamed *Americamysis bahia.* Results indicate increased toxicity because of low DO in renewal concentrations since renewal water was not aerated prior to use

[6]*Mysidopsis bahia* renamed *Americamysis bahia.*

ATTACHMENT I

Chain-of Custody

CH2MHILL Applied Sciences Lab
CHAIN OF CUSTODY RECORD
AND AGREEMENT TO PERFORM SERVICES

CVO 2300 NW Walnut Boulevard
Corvallis, OR 97330-3638
(541) 752-4271 FAX (541) 752-0276

ESI # 9919

COC #

Project # 147323.JC.01.TW	Purchase Order #
Project Name SAMOA JCO - 2001 TRADEWIND	
Company Name CH2M HILL	
Project Manager or Contact & Phone # STEVE COSTA 707-677-0123	Report Copy to: SAME
Requested Completion Date:	Site ID
Sample Disposal: Dispose ☒ Return ☐	

Sampling Date	Sampling Time	Type COMP	Type GRAB	Matrix WATER	Matrix SOIL	Matrix AIR	CLIENT SAMPLE ID (9 CHARACTERS)	LAB QC	TOTAL # OF CONTAINERS	Requested Analytical Method # MYSID ACUTE BIOASSAY	Preservative
24-25 OCT 2001		X		X			JCO-01-TW		1	X	

THIS AREA FOR LAB USE ONLY

Lab #	Page	of
Lab PM	Custody Review	
Log In	LIMS Verification	
pH	Custody Seals Y N	
	Ice Y N	

QC Level 1 2 3 Other

Cooler Temperature

Alternate Description	Lab ID

Relinquished By	Date/Time	Received By Empty Bottles	Date/Time
Sampled By and Title CLIENT (Please sign and print name)	Date/Time 25OCT01	Relinquished By STEVE COSTA (Please sign and print name)	Date/Time 26 OCT 01
Received By (Please sign and print name)	Date/Time	Relinquished By (Please sign and print name)	Date/Time
Received By (Please sign and print name)	Date/Time	Shipped Via UPS Fed-Ex Other DHL	Shipping #

Special Instructions: SEE PREVIOUS INSTRUCTIONS

ESI SAMPLE RECEIPT RECORD

[illegible]
[illegible]
[illegible]
[illegible]

ESI STUDY NUMBER: 9919

SAMPLE RECEIPT DATE: 11/1/01 TIME: 1352

SAMPLE RECEIVED BY: BC

DELIVERED VIA: ☐ FEDEX ☐ CLIENT ☐ ESI ☐ UPS ☑ OTHER

SAMPLE CONDITION:

CHAIN OF CUSTODY:	☑ YES	☐ NO
CHAIN OF CUSTODY SIGNED:	☑ YES	☐ NO
CHAIN OF CUSTODY COMPLETE:	☐ YES	☑ NO
SAMPLE DATE:	☑ YES	☐ NO
SAMPLE TIME RECORDED:	☐ YES	☑ NO
SAMPLE TYPE IDENTIFIED:	☑ YES	☐ NO
CUSTODY SEAL IN PLACE:	☑ YES	☐ NO
SHIPPING CONTAINER INTACT:	☑ YES	☐ NO

SAMPLE TEMPERATURE (AT ARRIVAL): 16° °C

COMMENTS:

COOLER NUMBER: _____

ATTACHMENT II

ESI Laboratory Report

TOXICOLOGICAL EVALUATION OF A TREATED EFFLUENT: BIOMONITORING SUPPORT FOR A NPDES PERMIT NOVEMBER 2001

American Samoa Joint Cannery Outfall

Prepared For

CH2M Hill, Incorporated
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Trinidad, California 95570-1238

By

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One Lafayette Road
Hampton, New Hampshire 03842

November 2001
Reference Number CH2M9919-01-11

STUDY NUMBER 9919

EXECUTIVE SUMMARY

The following summarizes the results of acute exposure bioassays performed from November 1-5, 2001 in support of the NPDES biomonitoring requirements of the American Samoa Joint Cannery Outfall. The 96 hour acute definitive assays were conducted using the marine species, *Americamysis bahia.*

Acute Toxicity Evaluation

Species	Exposure	LC-50	NOEC	LOEC
Americamysis bahia	24-Hours	46.78%	25%	50%
	48-Hours	44.86%	25%	50%
	72-Hours	37.52%	25%	50%
	96-Hours	37.52%	25%	50%

COMMENTS:
Results reflect test concentrations after salinity adjustment. See Section 2.3.

Authorized Signature: _____ 11/05/07

President ~ EnviroSystems, Incorporated Date

TOXICOLOGICAL EVALUATION
OF A TREATED EFFLUENT:
BIOMONITORING SUPPORT FOR A NPDES PERMIT
NOVEMBER 2001

American Samoa Joint Cannery Outfall

1.0 INTRODUCTION

Acute toxicity tests involve preparing a series of concentrations by diluting effluent with control water. Groups of test animals are exposed to each effluent concentration and a control for a specified period. In acute tests, mortality data for each concentration are used to calculate (by regression) the median lethal concentration, or LC-50, defined as the effluent concentration which kills half of the test animals. Samples with high LC-50 values are less likely to cause significant environmental impact. The acute no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) document the highest and lowest effluent concentrations that have no impact and a significant impact on the test species, respectively.

This report presents the results of acute toxicity tests conducted on an effluent sample collected from the American Samoa Joint Cannery Outfall. Testing was based on programs and protocols developed by the US EPA (1993) and involved conducting 96 hour acute static renewal toxicity tests with the marine species, *Americamysis bahia*. Testing was performed at EnviroSystems, Incorporated (ESI), Hampton, New Hampshire.

2.0 MATERIALS AND METHODS

2.1 General Methods

Toxicological and analytical protocols used in this program follow procedures primarily designed by the EPA to provide standard approaches for the evaluation of toxicological effects of discharges on aquatic organisms, and for the analysis of water samples. See Section 4.0 for a list of references.

2.2 Test Species

A. bahia, ≤5 days old, were from cultures maintained by Aquatic Research Organisms, Incorporated of Hampton, New Hampshire. Test organisms were transferred to test chambers by large bore pipet, minimizing the amount of water added to test solutions.

2.3 Effluent and Dilution Water

The effluent sample used in the assay was identified as "JC0-01-TW." Sample collection information is provided in Table 4. Upon receipt, the unused sample portion was stored at 4°C. All sample material used in the assay was warmed to test temperature prior to preparing test solutions. Total residual chlorine (TRC) was measured using amperometric titration (MDL 0.05 mg/L). As the effluent sample contained <0.05 mg/L TRC, dechlorination with sodium thiosulfate was not required (EPA 1993). Subsamples of undiluted effluent were collected for ammonia analysis upon arrival and again prior to renewal. At arrival, the effluent sample had a salinity of 9‰. Salinity of the effluent was increased to 24‰ by the addition of 35 grams of artificial sea salts to 2000 mL of effluent. Test concentrations for the assay were 50%, 25%, 12.5%, 6.25% and 3.1% effluent with dilution water control.

The dilution water used in this assay was obtained by ESI from its sea water system. The water is pumped in from the Hampton Estuary on the flood tide, filtered through a high volume sand filter, and stored in 3000 gallon polyethylene tanks. The water is classified as Class A waters by the State of New Hampshire and has been used for culture of test organisms for over 20 years. Sea water used in the assay had a salinity of 25‰ and a TRC of <0.05 mg/L.

2.4 Acute Toxicity Tests

The 96 hour acute static renewal toxicity tests were conducted at 25±1°C with a photoperiod of 16:8 hours light:dark. Test chambers for the acute assays were 250 mL glass beakers containing 200 mL test solution in each of 5 replicates with 10 organisms/replicate. Survival, dissolved oxygen, pH, salinity and temperature were measured daily in all replicates. Test solutions were renewed after 48 hours exposure with effluent from the start sample. Mysid shrimp were fed <24 hour old brine shrimp on a daily basis.

2.5 Data Analysis

Survival data at 24 hour intervals were analyzed to assess toxicity using a program called TOXSTAT® which can compute LC-50 values using the Spearman-Karber and Probit computation methods. If survival in the highest test concentration was >50%, LC-50 values were obtained by direct observation of the raw data. The NOEC was determined as the highest test concentration which caused no significant mortality while the LOEC was determined as the lowest concentration that did cause significant
mortality.

2.6 Quality Control

As part of the laboratory quality control program, standard reference toxicant assays are conducted on a regular basis for each test species. These results provide relative health and response data while allowing for comparison with historic data sets. A forty-eight hour acute reference toxicant assay was performed with *A. bahia* on October 31, 2001. Results of this assay were within one standard deviation of the historic mean for the species. See Table 2 for details.

2.7 Protocol Deviations

Two deviations from protocol was observed during these assays. Protocol requires testing temperatures to be maintained at 20°C ±2°C. Actual testing temperatures ranged from 21°C to 24°C. Ammonia was analyzed on 100% effluent only, not on 50% effluent which was the highest concentration tested. It is the opinion of the study director that these deviations from protocol had no impact on the outcome of the assay.

3.0 RESULTS

Results of the acute exposure bioassay conducted using the mysid shrimp are summarized in Table 1. A summary of reference toxicant data for the test species is presented in Table 2. Effluent and dilution water characteristics are presented in Table 3. Sample collection information is provided in Table 4. Table 5 provides a summary of historic data associated with the discharge. Support data are included in Appendix A.

3.1 Acute Toxicity Test - *Americamysis bahia*

There was 96% survival in the laboratory diluent control after 96 hours exposure. These results are an indication of healthy test organisms and that the dilution water had no adverse impact on the outcome of the assay.

Table 1 provides a summary of the acute exposure data and results.

3.2 Summary

The salinity adjusted effluent sample for the American Samoa Joint Cannery site exhibited signs of acute toxicity to the mysid shrimp, *Americamysis bahia,* during the 96 hour exposure period.

4.0 LITERATURE CITED

APHA. 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th edition. Washington D.C.

Stephan, C. 1982. Documentation for Computing LC-50 Values with a Mini Computer. Unpublished.

US EPA. 1993. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms.* Fourth Edition. EPA/600/4-90/027F.

US EPA. 2000. *Attachment G: NPDES Whole Effluent Toxicity Testing, Monitoring and Reporting Tips and Common Pitfalls.* Dated December 2000. US EPA Region I Offices, Boston, Massachusetts.

TABLE 1. Summary of Acute Evaluation Results. American Samoa Joint Cannery Outfall Effluent Evaluation. November 2001.

Concentration % Effluent	Exposure	Replicates A	B	C	D	E	Mean	Standard Deviation	Coefficient of Variation
Lab Control	Start	10	10	10	10	10			
	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	8	10	10	10	10	96%	0.894	93.17%
3.1%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	10	10	10	10	100%	0.000	0.00%
6.25%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	10	10	10	10	100%	0.000	0.00%
12.5%	24-Hours	10	10	10	10	10	100%	0.000	0.00%
	48-Hours	10	10	10	10	10	100%	0.000	0.00%
	72 Hours	10	10	10	10	10	100%	0.000	0.00%
	96-Hours	10	10	10	10	10	100%	0.000	0.00%
25%	24-Hours	10	10	10	10	9	98%	0.447	45.63%
	48-Hours	10	9	10	10	9	96%	0.548	57.05%
	72 Hours	10	9	10	10	9	96%	0.548	57.05%
	96-Hours	10	9	10	10	9	96%	0.548	0.00%
50%	24-Hours	0	10	1	0	8	38%	4.817	1267.54%
	48-Hours	0	8	1	0	8	34%	4.219	1240.88%
	72 Hours	0	0	1	0	1	4%	0.548	0.00%
	96-Hours	0	0	1	0	1	4%	0.548	0.00%

SUMMARY OF ENDPOINTS

Exposure Period	LC-50	NOEC	LOEC
24-Hours	46.78% (43-50)	25.0%	50.0%
48-Hours	44.86% (41-49)	25.0%	50.0%
72-Hours	37.52% (34-41)	25.0%	50.0%
96-Hours	37.52% (34-41)	25.0%	50.0%

TABLE 2. Summary of Reference Toxicant Data. American Samoa Joint Cannery Outfall Effluent Evaluation. November 2001.

Concentrations Expressed as mg/L Sodium Dodecyl Sulfate

Species	Date	LC-50	Historic Mean	Number of Tests	±1 STD Deviation	±2 STD Deviations
A. bahia	10/31/01	23.4	19.8	130	4.36	8.73

TABLE 3. Summary of Effluent and Diluent Characteristics. American Samoa Joint Cannery Outfall Effluent Evaluation. November 2001.

Parameter	Units	EFFLUENT	DILUENT
Salinity - on Arrival	‰	9	25
After Salinity Adjustment ‡	‰	24	-
pH - on Arrival	SU	6.56	8.15
After Salinity Adjustment ‡	SU	7.17	-
TRC	mg/L	<0.05	<0.05
Dissolved Oxygen	mg/L	1.2	7.4
Ammonia - at Start	mg/L as N	56.1	<0.10
Unionized Ammonia	mg/L as N	0.087	-
Ammonia - at 48 Hours	mg/L as N	52.6	<0.10
Unionized Ammonia	mg/L as N	0.380	-

TABLE 4. Summary of Sample Collection Information. American Samoa Joint Cannery Outfall Effluent Evaluation. November 2001.

Sample Description	Type	Collection Date	Collection Time	Receipt Date	Receipt Time	Arrival Temp °C
EFFLUENT	Comp	10/24-25/01	ND	11/01/01	1350	10

COMMENTS:
‡ - Recorded in the 50% effluent concentration, not 100% salinity adjusted effluent.
ND - No data was recorded on chain of custody.

TABLE 5. Summary of StarKist Samoa and COS Samoa Packing Combined Effluent Bioassay Results. American Samoa Joint Cannery Outfall Effluent Evaluation. November 2001.

Date	Species	96-Hour Endpoints		
		LC-50	NOEC	LOEC
02/93[1]	*Penaeus vannami*	4.8%	3.1%	6.25%
10/93[1]	*Penaeus vannami*	15.67%	3.1%	6.25%
02/94[1]	*Penaeus vannami*	15.76%	<1.6%	1.6%
10/94[1]	*Americamysis bahia*	31.2%	25.0%	50.0%
11/95[1]	*Penaeus vannami*	14.8%	6.25%	12.5%
11/95[1]	*Americamysis bahia*	10.8%	6.25%	12.5%
02/96[1]	*Penaeus vannami*	>50.0%	>50.0%	>50.0%
11/96[1]	*Penaeus vannami*	44.4%	25.0%	50.0%
11/96[1]	*Penaeus vannami*	7.11%	3.1%	6.25%
11/97[1]	*Penaeus vannami*	39.36%	12.5%	25.0%
09/97[1]	*Penaeus vannami*	12.3%	6.25%	12.5%
06/98[1]	*Americamysis bahia*	17.2%	6.25%	12.5%
11/98[1]	*Americamysis bahia*	15.0%	6.25%	12.5%
02/00[1]	*Americamysis bahia*	20.0%	6.25%	12.5%
08/00[1]	*Americamysis bahia*	17.1%	3.1%	6.25%
05/01[2]	*Americamysis bahia*	13.81%	12.5%	25.0%
11/01[2]	*Americamysis bahia*	37.52%	25.0%	50.0%

Notes:

1. Assays conducted by Advanced Biological Testing, Inc., Rohnert Park, California. Results were provided by CH2M Hill.

2. Assay conducted by EnviroSystems, Inc., Hampton, New Hampshire

APPENDIX A

DATA SHEETS

STATISTICAL SUPPORT

METHODS USED IN NPDES PERMIT BIOMONITORING TESTING

Parameter	Method
Acute Exposure Bioassays	
Ceriodaphnia dubia, Daphnia pulex	EPA 600/4-90/027
Pimephales promelas	EPA 600/4-90/027
Americamysis bahia	EPA 600/4-90/027
Menidia beryllina, Cyprinodon variegatus	EPA 600/4-90/027
Chronic Exposure Bioassays	
Ceriodaphnia dubia	EPA 600/4-91/002, 1002.0
Pimephales promelas	EPA 600/4-91/002, 1000.0
Cyprinodon variegatus	EPA 600/4-91/003, 1004.0
Menidia beryllina	EPA 600/4-91/003, 1006.0
Arbacia punctulata	EPA 600/4-91/003, 1008.0
Champia parvula	EPA 600/4-91/003, 1009.0
Trace Metals:	
ICP Metals	EPA 200.7/SW 6010
Hardness	Standard Methods 20th Edition - Method 2340 B
Wet Chemistries:	
Alkalinity	Standard Methods 20th Edition - Method 310.1
Chlorine, Residual	Standard Methods 20th Edition - Method 4500CLD
Total Organic Carbon	Standard Methods 20th Edition - Method 5310.6
Specific Conductance	Standard Methods 20th Edition - Method 2510B
Nitrogen - Ammonia	Standard Methods 20th Edition - Method 4500NH3G
pH	Standard Methods 20th Edition - Method 4500H+B
Solids, Total (TS)	Standard Methods 20th Edition - Method 2540.B
Solids, Total Suspended (TSS)	Standard Methods 20th Edition - Method 2540D
Dissolved Oxygen	Standard Methods 20th Edition - Method 4500-O G

ACUTE BIOASSAY DATA SUMMARY

* Pull from Sal Adj ? ma ... ? BC

STUDY: 9919	SAMPLE RECEIVED:	"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES
CLIENT: CH2M Hill	TEST ORGANISM: *A. bahia*	
SAMPLE: American Samoa	ORGANISM SUPPLIER:	
DILUENT: LAB SALT	ORGANISM BATCH/AGE:	

	TRC	AMM 0 HR✖	* AMM 48 HR✖	pH	DO	Salinity
EFFLUENT	<0.05	BC	BC	6.56	1.2	9.4
DILUENT	<0.05	BC	BC	8.08	7.0	24

SALINITY ADJUSTMENT RECORD (IF APPLICABLE): 2000 ML EFFLUENT + 35 G SEA SALTS = 100% ACTUAL PERCENTAGE

CONC	REP	SURVIVAL 0	24	48	72	96	✦DISSOLVED OXYGEN (MG/L)✦ 0	24	48◊	48☆	72	96
LAB	A	10	10	10	10	8	7.4	7.0	7.0	7.0	7.5	6.9
	B	10	10	10	10	10	7.5	6.9	6.8	7.0	7.6	7.1
	C	10	10	10	10	10	7.5	6.9	6.7	7.0	7.5	7.1
	D	10	10	10	10	10	7.5	6.8	6.7	7.0	7.3	7.1
	E	10	10	10	10	10	7.2	6.8	6.5	7.0	7.3	7.1
3.1%	A	10	10	10	10	10	7.5	6.9	6.5	7.0	7.4	7.1
	B	10	10	10	10	10	7.5	6.7	6.5	7.0	7.4	7.1
	C	10	10	10	10	10	7.5	6.6	6.6	7.0	7.2	7.0
	D	10	10	10	10	10	7.6	6.6	6.6	6.8	7.1	7.0
	E	10	10	10	10	10	7.5	6.7	6.6	6.7	7.0	7.1
6.25%	A	10	10	10	10	10	7.5	6.7	6.7	6.7	7.2	7.1
	B	10	10	10	10	10	7.0	6.6	6.7	6.5	7.2	7.1
	C	10	10	10	10	10	7.1	6.6	6.8	6.5	7.2	7.1
	D	10	10	10	10	10	7.2	6.5	6.7	6.5	7.1	7.0
	E	10	10	10	10	10	7.3	6.4	6.7	6.5	7.0	6.9
DATE		11/1/01	11/2/01	11/3	11/4	11/5/01	11/1	11/2/01	11/3	11/3	11/4	11/5/01
TIME		1645	1700	1530	1530	1555	1600	1700	1500	1600	1555	1555
INITIALS		✓	✓	BC	BSM	✓	BC	✓	BC	BC	BSM	✓
FED?		✓	✓	✓	✓		\			✓	✓	

CONC	REP	PH (SU) 0	24	48◊	48☆	72	96
LAB	A	8.15	7.96	8.01	8.03	8.20	8.18
	B	8.15	8.00	8.03	8.03	8.19	8.19
	C	8.15	7.99	8.02	8.02	8.17	8.19
	D	8.15	8.00	8.02	8.03	8.17	8.19
	E	8.15	8.04	7.94	8.03	8.20	8.20
3.1%	A	8.06	7.89	8.25	8.00	8.17	8.19
	B	8.06	7.92	8.01	7.97	8.18	8.20
	C	8.06	7.89	8.02	7.96	8.15	8.20
	D	8.06	7.95	8.02	7.97	8.18	8.19
	E	8.06	7.93	8.02	7.97	8.18	8.19
6.25%	A	7.96	7.92	8.09	7.91	8.14	8.20
	B	7.96	7.91	8.09	7.94	8.16	8.22
	C	7.96	7.90	8.10	7.91	8.19	8.22
	D	7.96	7.87	8.07	7.94	8.15	8.21
	E	7.91	7.90	8.05	7.91	8.15	8.21
DATE		11/1	11/2/01	11/3 →		11/4	11/5/01
TIME		1600	1700	1500	1600	1555	1555
INITIALS		BC	✓	BC	BC	BSM	✓
FED?		\			X ✓		

CONC	REP	TEMPERATURE (°C) 0	24	48◊	48☆	72	96
LAB	A	22	24	24	23	24	24
	B	23	24	24	23	24	24
	C	23	24	24	23	24	24
	D	23	24	24	23	24	24
	E	22	24	24	23	24	24
3.1%	A	23	24	24	23	24	24
	B	23	24	24	23	24	24
	C	23	24	24	23	24	24
	D	23	24	24	23	24	24
	E	23	24	24	23	24	24
6.25%	A	23	24	24	23	24	24
	B	23	24	24	23	24	24
	C	23	24	24	23	24	24
	D	23	24	25	23	24	24
	E	23	24	24	23	24	24
DATE		11/1	11/2/01	11/3 →		11/4	11/5/01
TIME		1600	1700	1500	1600	1555	1555
INITIALS		BC	✓	BC →		BSM	✓
FED?		\				✓	

CONC	REP	SALINITY (ppt) 0	24	48◊	48☆	72	96
LAB	A	25	25	26	25	27	28
	B	25	27	29	25	27	29
	C	25	27	29	26	27	30
	D	25	26	28	26	27	29
	E	25	26	28	26	27	29
3.1%	A	25	27	29	26	28	29
	B	25	26	28	26	27	29
	C	25	26	28	26	28	28
	D	25	27	28	26	27	29
	E	25	27	28	26	27	30
6.25%	A	25	27	29	26	27	29
	B	25	27	28	26	27	29
	C	25	27	28	26	26	29
	D	25	27	28	26	27	29
	E	25	27	29	26	27	30
DATE		11/1	11/2/01	11/3 →		11/4	11/5/01
TIME		1600	1700	1500	1600	1555	1555
INITIALS		BC	✓	BC →		BSM	✓
FED?		\				✓	

✖ - Pull on 50% effluent also.
◊ - "Old" water qualities (prior to renewal)
✦ - AERATE FROM START!
☆ - "New" water qualities (post renewal)

ACUTE BIOASSAY DATA SUMMARY

STUDY: 9919	SAMPLE RECEIVED:	"AS RECEIVED" EFFLUENT AND DILUENT CHEMISTRIES						
CLIENT: CH2M Hill	TEST ORGANISM: *A. bahia*		TRC	AMM 0 HR✖	AMM 48 HR✖	pH	DO	Salinity
SAMPLE: American Samoa	ORGANISM SUPPLIER:	EFFLUENT	See page 1 for details and salinity adjustment record.					
DILUENT: LAB SALT	ORGANISM BATCH/AGE:	DILUENT						

CONC	REP	SURVIVAL 0	24	48	72	96	✦DISSOLVED OXYGEN (MG/L)✦ 0	24	48◊	48☆	72	96
12.5%	A	10	10	10	10	10	7.2	6.1	6.8	6.0	7.0	6.9
	B	10	10	10	10	10	7.2	6.1	6.8	5.8	6.9	6.9
	C	10	10	10	10	10	7.1	5.7	6.9	5.8	6.8	6.8
	D	10	10	10	10	10	7.2	5.8	7.0	6.0	6.9	6.9
	E	10	10	10	10	10	6.8	6.1	7.0	6.0	7.0	6.9
25%	A	10	10	10	10 [illegible]	10	6.8	5.2	6.2	5.5	6.8	7.0
	B	10	10	9	9 [illegible]	9	6.3	3.4	6.0	5.5	4.7	4.7
	C	10	10	9 10	9 10	10	6.6	5.2	6.5	5.3	6.7	6.8
	D	10	10	10	10	10	6.6	5.3	6.6	5.2	6.8	4.1
	E	10	9	9	9	9	6.6	5.1	6.6	5.2	6.5	4.3
50%	A	10	0*	\	→	→	6.7	0.5	\	→	→	→
	B	10	10	8	0 →	→	5.6	4.3	6.0	5.3	5.0 →	→
	C	10	1	1	1	1	5.5	5.0	6.5	5.1	5.4	6.6
	D	10	0	\	→	→	5.5	5.1	\	→	→	→
	E	10	8	8	(11) 8 [illegible]	(11) [illegible]	5.7	4.6	6.5	5.0	5.8	4.0
DATE		11/1/01	11/2/01	11/3	11/4	11/5/01	11/1	11/2/01	11/3 →		11/4	11/5/01
TIME		1645	1700	1530	1550	1555	1600	1700	1500	1600	1535	1555
INITIALS		✓	✓	BC	RSM	✓	BC	✓	BC →		RSM	✓
FED?		/			✓		NO				✓	

CONC	REP	PH (SU) 0	24	48◊	48☆	72	96	TEMPERATURE (°C) 0	24	48◊	48☆	72	96
12.5%	A	7.79	7.92	8.10	7.84	8.20	8.21	22	24	24	23	24	24
	B	7.79	7.90	8.10	7.84	8.19	8.23	22	24	24	23	24	24
	C	7.79	7.83	8.10	7.84	8.17	8.24	22	24	24	23	24	24
	D	7.78	7.96	8.16	7.83	8.15	8.26	22	24	25	23	24	24
	E	7.78	7.84	8.10	7.84	8.16	8.22	22	24	24	23	24	24
25%	A	7.51	8.03	8.04	7.64	8.29	8.34	22	24	24	23	24	24
	B	7.50	7.72	8.21	7.70	8.29	7.99	22	24	24	23	24	24
	C	7.50	8.06	8.20	7.71	8.28	8.32	22	24	24	23	24	24
	D	7.50	7.91	8.17	7.63	8.20	7.90	22	24	24	24	24	24
	E	7.50	7.88	8.15	7.60	8.21	7.88	22	24	24	24	24	24
50%	A	7.17	7.82	\	→	→	→	21	24	\	\	\	\
	B	7.17	8.13	8.36	7.62	8.06 →	→	21	24	24	24	24 →	→
	C	7.16	8.16	8.18	7.82	8.04	8.40	21	24	25	23	24	24
	D	7.16	8.02	\	→	→	→	21	24	\	~~23~~ →	→	→
	E	7.27	7.98	8.14	7.83	8.14	7.90	21	24	24	23	24	24
DATE		11/1	11/2/01	11/3 →		11/4	11/5/01	11/1	11/2/01	11/3 →		11/4	11/5/01
TIME		1600	1700	1500	1600	1600	1555	1600	1700	1500	1600	1600	1555
INITIALS		BC	✓	BC →		RSM	✓	BC	✓	BC →		RSM	✓
FED?		N				✓		N				✓	

CONC	REP	SALINITY (ppt) 0	24	48◊	48☆	72	96
12.5%	A	25	27	28	26	27	[illegible]
	B	25	27	29	26	27	[illegible]
	C	25	26	28	26	27	[illegible]
	D	25	26	28	26	27	[illegible]
	E	25	26	28	26	27	30
25%	A	25	27	28	25	27	30
	B	25	27	28	25	27	30
	C	25	27	28	25	27	30
	D	25	26	28	25	27	28
	E	25	26	28	25	27	30
50%	A	24	26	\	\	\	\
	B	24	26	22	25	27	→
	C	24	26	27	26	27	29
	D	24	26	\	~~24~~ →	→	→
	E	24	26	27	26	27	29
DATE		11/1	11/2/01	11/3 →		11/4	11/5/01
TIME		1600	1700	1500	1600	1600	1555
INITIALS		BC	✓	BC →		RSM	✓
FED?		\				✓	

✖ - Pull on 50% effluent also.
◊ - "Old" water qualities (prior to renewal)

✦ - AERATE FROM START!
☆ - "New" water qualities (post renewal)

*[illegible]

Title: 9919 American Samoa: A. bahia Survival 48 hours
File: 9919ab48sv Transform: NO TRANSFORMATION

Probit Analysis - not Using Smoothed Proportions

DOSE	NUMBER SUBJECTS	NUMBER OBSERVED	OBSERVED PROPORTION	PREDICTED PROPORTION
3.10	50	50	1.0000	0.9997
6.25	50	50	1.0000	0.9993
12.50	50	50	1.0000	0.9964
25.00	50	47	0.9400	0.9503
50.00	50	17	0.3400	0.3351

Est. Mu = 44.8640 Est. Sigma = 12.0561
sd = 1.8909 sd = 1.7075

Chi-Square lack of fit = 0.3471 Likelihood lack of fit = 0.5694
Table Chi-square = 11.3449 (alpha = 0.01, df = 3)
Table Chi-square = 7.8147 (alpha = 0.05, df = 3)

Title: 9919 American Samoa: A. bahia Survival 48 hours
File: 9919ab48sv Transform: NO TRANSFORMATION

Probit EC Estimates
WITHOUT CONTROL DATA

POINT	EST. END POINT	95% CONFIDENCE LIMITS	
EC 1	16.8173	9.0754	24.5591
EC 5	25.0334	19.2156	30.8513
EC10	29.4135	24.4951	34.3318
EC20	34.7173	30.6609	38.7737
EC25	36.7323	32.9045	40.5600
EC30	38.5418	34.8585	42.2250
EC40	41.8096	38.2201	45.3991
EC50	44.8640	41.1579	48.5701
EC60	47.9184	43.9154	51.9214
EC70	51.1862	46.7069	55.6656
EC75	52.9957	48.1989	57.7926
EC80	55.0107	49.8258	60.1956
EC90	60.3146	53.9845	66.6446
EC95	64.6946	57.3306	72.0586
EC99	72.9107	63.4894	82.3321

Title: 9919 American Samoa: A. bahia 72 & 96 Hr Survival
File: 9919ab72sv Transform: NO TRANSFORMATION

Probit Analysis - not Using Smoothed Proportions

DOSE	NUMBER SUBJECTS	NUMBER OBSERVED	OBSERVED PROPORTION	PREDICTED PROPORTION
3.10	50	50	1.0000	1.0000
6.25	50	50	1.0000	1.0000
12.50	50	50	1.0000	0.9998
25.00	50	48	0.9600	0.9606
50.00	50	2	0.0400	0.0398

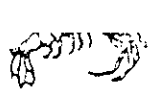
Est. Mu = 37.5160 Est. Sigma = 7.1217
sd = 1.6173 sd = 0.9103

Chi-Square lack of fit = 0.0119 Likelihood lack of fit = 0.0233
Table Chi-square = 11.3449 (alpha = 0.01, df = 3)
Table Chi-square = 7.8147 (alpha = 0.05, df = 3)

Title: 9919 American Samoa: A. bahia 72 & 96 Hr Survival
File: 9919ab72sv Transform: NO TRANSFORMATION

Probit EC Estimates
WITHOUT CONTROL DATA

POINT	EST. END POINT	95% CONFIDENCE LIMITS	
EC 1	20.9486	15.7626	26.1345
EC 5	25.8019	21.5135	30.0904
EC10	28.3892	24.5077	32.2707
EC20	31.5223	28.0344	35.0101
EC25	32.7125	29.3382	36.0868
EC30	33.7814	30.4893	37.0735
EC40	35.7117	32.5162	38.9073
EC50	37.5160	34.3460	40.6860
EC60	39.3202	36.1117	42.5288
EC70	41.2506	37.9324	44.5688
EC75	42.3195	38.9124	45.7266
EC80	43.5097	39.9823	47.0371
EC90	46.6428	42.7072	50.5783
EC95	49.2301	44.8788	53.5813
EC99	54.0834	48.8240	59.3429



Aquatic Research Organisms

Lot # 10301ABARO
10/31/01 pm

DATA SHEET

I. Organism History

Species: America mysis bahia

Source: Lab reared ✓ Hatchery reared _____ Field collected _____

Hatch date 10/29/01 Receipt date _____

Lot number 10290/MS Strain ARO

Brood Origination FL

II. Water Quality

Temperature 25 °C Salinity 28 ppt DO Sat

pH 7.8 Hardness — ppm

III. Culture Conditions

System: Sw heated recirc.

Diet: Flake Food ✓ Phytoplankton _____ Trout Chow _____

Brine Shrimp ✓ Rotifers _____ Other _____

Prophylactic Treatments: _____

Comments: _____

_____

IV. Shipping Information

Client: EST # of Organisms: 600

Carrier: Pick up Date Shipped: 10/31/01

Biologist: Alan Lantzke

1 - 800 - 927 - 1650

PO Box 1271 • One Lafayette Road • Hampton, NH 03842 • (603) 926-1650

PREPARATION OF DILUTIONS

STUDY: 9919	CLIENT: CH2M HILL - American Samoa
SPECIES: *A. bahia*	

Diluent: Lab Salt	Day: 0 start Sample: EOB Labsalt		Day: 2 Sample: EOB					
Concentration	Vol. Eff.	Final Vol	Vol. Eff.	Final Vol	HRS	Date	Time	Initials
LAB	0	1000	0	750	0	11/1	1530	BC
3.1%	31.0	↓	23.4	↓	48			
6.25%	62.5		46.9		Comments:			
12.5%	125		93.75					
25%	250		187.5					
50%	500	↓	375	↓				

RECORD OF METERS USED FOR WATER QUALITY MEASUREMENTS

STUDY: 9919	CLIENT: CH2M HILL - American Samoa					
WATER QUALITIES - A. bahia						
HOURS:	0	24	48 - old	48 - new	72	96
Water Quality Station #	1	2	2	2	1	2
Initials	BC		BC	BC	RSMC	
Date	11/1/01	11/2/01	11/3/01	11/3	11/4	11/5/01

Water Quality Station #1		Water Quality Station #2		COMMENTS
DO meter #	21	DO meter #	19	
DO probe #	3	DO probe #	1	
pH meter #	1138	pH meter #	56	
pH probe #	27	pH probe #	26	
S/C meter #	YSI30	S/C meter #	YSI30	
S/C probe #	NA	S/C probe #	NA	
Salinity meter #	YSI30	Salinity meter #	YSI30	

CH2MHILL

Steve Costa
Karen Glatzel

216 Driftwood Lane
P.O. Box 1238
Trinidad, CA 95570-1238

707-677-0123 (Vox)
707-677-9210 (Fax)
510-508-5020 (Cell)

8 January 2002

Mr. Carl Goldstein
Pacific Insular Area Programs
CMD-1
Environmental Protection Agency
75 Hawthorne Street
San Francisco, CA 94105

Mr. Peter Peshut
American Samoa Environmental Protection Agency
American Samoa Government
P.O. Box 368A
Pago Pago, American Samoa 96799

Enclosed is the required report for the bioassay test results for the October 2001 effluent sampling for Joint Cannery Outfall in American Samoa. The sampling and analysis were carried out without problems. The result are similar to the past bioassay test results.

Please call us if you have any questions or comments on the enclosed report,

Sincerely,

Steven L. Costa

Cc: Jim Cox, COS International; Herman Gebauer, COS; Brett Ransby, COS; John Brown, Heinz; Phil Thirkel, StarKist Samoa; Joe Carney, StarKist Samoa; David Wilson, CH2M HILL.

Encl: Effluent Bioassay Results for October 2001 Sampling